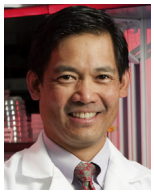
Gastroenterology 2014;146:1547–1553

Role of the Intestinal Microbiota in Resistance to Colonization by *Clostridium difficile*

Robert A. Britton[1] Vincent B. Young[2,3]

[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan; [2]Division of Infectious Diseases, Department of Internal Medicine, [3]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan

Antibiotic-associated infection with the bacterial pathogen *Clostridium difficile* is a major cause of morbidity and increased health care costs. *C difficile* infection follows disruption of the indigenous gut microbiota by antibiotics. Antibiotics create an environment within the intestine that promotes *C difficile* spore germination, vegetative growth, and toxin production, leading to epithelial damage and colitis. Studies of patients with *C difficile* infection and animal models have shown that the indigenous microbiota can inhibit expansion and persistence of *C difficile*. Although the specific mechanisms of these processes are not known, they are likely to interfere with key aspects of the pathogen's physiology, including spore germination and competitive growth. Increasing our understanding of how the intestinal microbiota manage *C difficile* could lead to better means of controlling this important nosocomial pathogen.

Keywords: Colonization Resistance; *C difficile*; Microbiome; Microbial Ecology; Antibiotics.

The fulfillment of Koch's postulates for *Clostridium difficile* in 1977 was one of the first formal recognitions of a microbiome-related disease.[1] Although *C difficile* itself fit into the mold of a typical bacterial pathogen, *C difficile* infection (CDI) was unique in that previous antibiotic treatment of the patient was necessary for development of the full disease phenotype. We review *C difficile* as a pathogenic organism and discuss the basic epidemiologic features of CDI, as well as its molecular pathogenesis. Research into the developing story of how *C difficile* interacts with the indigenous intestinal microbiota has provided important insights into the role of the microbiome in human health and disease.

C difficile and Antibiotic-Associated Colitis

C difficile is a gram-positive, anaerobic, spore-forming bacterium that was first isolated from the feces of healthy infants.[2] Interestingly, the organism appears to be highly prevalent in infants, who rarely show any clinical signs of infection with fully virulent strains.[3,4] As the child and subsequently the microbiome matures, *C difficile* is no longer readily detected; in the healthy adult population, asymptomatic colonization with this organism is considered to be a rare event.[4,5] Rates of colonization are increased primarily among adults with frequent health care–associated contact and patients in chronic-care facilities.[5]

The pathogenesis of CDI involves production of 2 members of the family of large clostridial toxins: TcdA and TcdB, which are products of genes located with a pathogenicity locus.[6–8] In *C difficile*, these toxins are large proteins with glycosyltransferase activities targeting the host guanosine triphosphatases Rho, Rac, and Cdc42, which regulate actin.[9,10] Administration of purified toxin in model systems, including ligated ileal loops, is sufficient to replicate the intestinal damage that is characteristic of CDI.[11] Furthermore, there is evidence that these toxins have systemic effects on the host.[12] Although TcdA and TcdB are the most well-studied virulence factors of *C difficile*, other putative virulence factors also have been described.[13] A subset of *C difficile* strains produce an additional toxin, often referred to as binary toxin, that is related to iota toxin from *Clostridium perfringens*.[14,15] Recent interest in binary toxin likely is related to the fact that the recent epidemic strains of *C difficile*, exemplified by the NAP1/027/BI strain, often produce this putative virulence factor.[16]

The epidemiology of CDI has been characterized by increases in incidence and severity that began around the year 2000.[17,18] It recently was estimated that CDI is the most

Abbreviations used in this paper: **CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2014.01.059

common health care–associated infection, producing estimated annual hospital costs of more than $3 billion.[19] The increase in virulence has been associated with the increasing isolation of epidemic strains related to NAP1/027/BI.[16,20] There has been debate as to whether epidemic strains have enhanced virulence properties that result in more severe disease or if these strains simply cause more cases of CDI without causing worse patient outcomes.[21] However, the overall increase in incidence and severity of CDI in the past decade has spurred research into *C difficile* pathogenesis. During the same period of time, the growing interest in the role of the indigenous microbiota in human health and disease, exemplified by projects such as the National Institutes of Health's Human Microbiome Project[22,23] and the European Metagenomics of the Human Intestinal Tract consortium,[24] has produced a scientific environment well suited for the study of CDI. There is now much interest in the interactions between this pathogen in the intestinal microbiome.

The Microbiota Affects *C difficile* Invasion

The concept that the indigenous gut microbiota mediates some form of resistance against colonization by bacterial pathogens was proposed long before *C difficile* was associated with antibiotic-associated pseudomembranous colitis.[25] Productive infection of animals with enteric bacterial pathogens often requires pre-exposure to antibiotics.[26–28] However, until recently, the specific mechanisms by which the indigenous microbiome could prevent colonization by pathogenic organisms were not of widespread scientific interest. The requirement for antibiotic treatment before infection with a pathogen was regarded simply as a technical hurdle to overcome to investigate specific interactions between the pathogen in the host. However, increasing interest in the role of the indigenous microbiome in maintaining intestinal homeostasis has focused attention on its interactions with pathogens. The development of tractable animal models for studying CDI and the concurrent application of experimental microbial ecology to host-associated bacterial communities has led to multiple reports of how they influence the development of CDI and its complications. Work in experimental models of CDI and with clinical material from patients has indicated that *C difficile*, the intestinal microbiota, and other host factors form a complex system; specific alterations to this system can promote pathogenesis of CDI (Figure 1).

Studies in animals and human beings have shown that antibiotics have profound and, in some cases, long-lasting effects on the community structure of the gut microbiota.[29,30] Antibiotics affect not only the overall size of the gut bacterial community, but also the composition of the community (they alter proportions of specific bacterial species). However, these effects do not necessarily occur

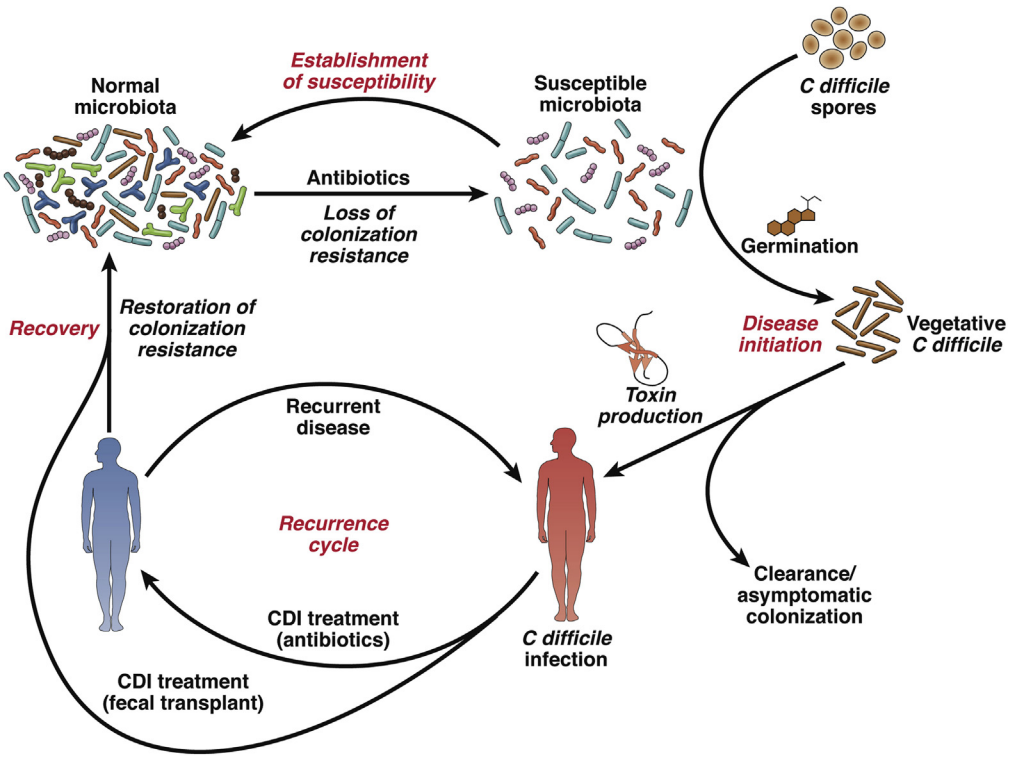


Figure 1. Cycle of CDI. Antibiotic administration alters the indigenous intestinal microbiota, producing an environment that permits germination of *C difficile* spores and expansion of the pathogen. *C difficile* produces toxins that cause colitis and resulting symptoms. Antibiotics directed against *C difficile* can decrease the load of the pathogen and toxin production. Returning the microbiota to a state of colonization resistance cures CDI. However, if the microbiota is unable to restore resistance to colonization by *C difficile*, then patients have recurring CDI. In certain cases, repeat courses of anti–*C difficile* antibiotic therapy can eradicate the pathogen. In other cases, therapeutic restoration of a diverse microbiota via FMT is required to overcome CDI.